CASWELL FILE 6

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

009417

APR 6 1992

PC 019401

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT **4-CHLOROPHENOXYACETIC ACID:** Phase V Review of Generic Data Submission. [ACTION 627].

FROM: Jess Rowland, M.S, Toxicologist Jess Rowland 3/31/92
Section II, Toxicology Branch II
Health Effects Division (H7509C)

TO: C. Rice / T. Luminello
Product Manager (52)
Reregistration Division

THRU: K.Clark Swentzel, Section Head K. Clark Swentzel 4/1/91
Section II, Toxicology Branch II
Health Effects Division (H7509C)
and
Marcia van Gemert, Ph.D., Chief Marcia van Gemert 4/2/92
Toxicology Branch II
Health Effects Division (H7509C)

STUDY IDENTIFICATIONS: Submission: S398032 Caswell No. 204

HED Project No. 1-1973 Registrant: La Choy Food Products

ACTION REQUESTED: Review of studies listed below:

1. Acute Oral Toxicity Study in Rats **[MRID No. 418370-01].**
2. Salmonella Mutagenicity Assay **[MRID No. 418370-02].**
3. In vivo Micronucleus Assay **[MRID No. 418370-03].**
4. Mouse Lymphoma Assay **[MRID No. 418370-04].**

RESPONSE: A separate Data Evaluation Report [DER] for each of the above mentioned studies is attached. A summary of each study is as follows:

1. Acute Exposure Oral Toxicity of 4-Chlorophenoxyacetic Acid in Rats [MRID No. 418370-01].

The estimated acute oral LD_{50} in male and female rats was determined to be 2703 mg/kg with 95% confidence limits of 2191 to 3335 mg/kg. **Toxicity Category III.** This study is core classified as supplementary due to the technical difficulties encountered. However, it is considered acceptable for guideline purposes since repeating the study will not alter the toxicity category. **This study does satisfy Guideline requirement for an acute oral toxicity study in rats 81-1.**

2. Mutagenicity Test on 4-Chlorophenoxyacetic Acid in the Salmonella/Mammalian-microsome Reverse Mutation Assay [Ames Test] with a Confirmatory Assay [MRID No. 418370-02].

When tested in the Salmonella/microsomal assay in strains TA98, TA100, TA 1535, TA1537 and TA1538, at concentrations ranging from 100 to 5000 µg/plate, 4-chlorophenoxyacetic acid was non mutagenic both in the presence and absence of metabolic activation. This study is classified as **Acceptable and therefore, satisfies the Guideline requirements for genetic effects Category I, Gene Mutations.**

3. Mutagenicity Test on 4-Chlorophenoxyacetic Acid In Vivo Micronucleus Assay [MRID No. 418370-03].

A single oral administration of 4-chlorophenoxyacetic acid at doses of 450, 900 or 1800 mg/kg to male and female mice did not cause a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow cells harvested 24, 48 and 72 hours posttreatment. Therefore, 4-CPA was not clastogenic under the conditions of this assay. This study is classified as **Acceptable and therefore, satisfies the Guideline requirements for genetic effects Category II, Structural Chromosomal Aberrations.**

4. Mutagenicity Test on 4-Chlorophenoxyacetic Acid in the L5178y $TK^{+/-}$ Mouse Lymphoma Forward Mutation Assay with Independent Repeat Tests [MRID No. 418370-04].

When tested in a in vitro mammalian assay, 4-CPA at doses ranging from 100 to 4000 µg/mL, did not induce forward mutations at the $TK^{+/-}$ locus in L5178Y mouse lymphoma cells in two independently performed trials. This study is classified as **Acceptable and therefore, satisfies the Guideline requirements for genetic effects Category I, Gene Mutations.**

Tox Chem No. 204 File Last Updated _____ Current Date _____

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA MRID NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
81-1 Acute Oral LD50 Species: Rat Hazleton; 00801531 02/28/91	4-CPA Acid 99%	418370-01	LD50 = 2703 mg/kg [95% confidence limits: 2191 - 3335 mg/kg]	III	Supplementary but acceptable RR
84-2(a) Gene Mutation Species: Salmonella Hazleton; 12447-0-401R 12/24/90	4-CPA Acid 99%	418370-02	Concentrations tested: 100, 333, 667, 1000, 3330 and 5000 µg/plate. Non mutagenic in Salmonella strains TA98, TA100, TA1535, TA1537 and TA1538 both with and without activation.	NA	Acceptable RR
84-2(b) In Vivo Micronucleus Species:Mice Hazleton; 12447-0-455PO 02/07/91	4-CPA Acid 99%	418370-03	No significant increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow cells of mice harvested 24, 48 and 72 hours following a single oral administration of 4-CPA at 450, 900 or 1800 mg/kg.	NA	Acceptable RR
84-1 In Vitro Mouse Lymphoma Species: L5178y $TK^{+/-}$ Hazleton; 12447-0-431 03/05/91	4-CPA Acid	418370-04	4-CPA Acid at doses ranging from 100 to 4000 µg/mL was not mutagenic with or without meatabolic activation.	NA	Acceptable RR

NEW INPUT
JR

DATA EVALUATION REPORT

4-Chlorophenoxyacetic Acid

Study Type: Acute Oral Toxicity in Rats

Study Title: Acute Exposure Oral Toxicity of 4-Chlorophenoxyacetic Acid in Rats

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

January 21, 1992

Principal Reviewer:	Betty Shindel Betty Shindel, M.P.H.	2-21-92 Date
Independent Reviewer:	John Liccione John Liccione, Ph.D.	2-21-92 Date
QA/QC Manager:	Sharon A. Segal Sharon Segal, Ph.D.	2-21-92 Date

Contract Number: 68D10075
Work Assignment Number: 1-062
Clement Number: 91-192
Project Officer: James E. Scott

EPA Reviewer: _____ 03/02/92
Dr. Jess Rowland Date
Review Section II, Toxicology Branch II (HED)

EPA Section Head: _____ 3/10/92
Dr. Clark Swentzel Date
Review Section II, Toxicology Branch II (HED)

DATA EVALUATION REPORT

STUDY TYPE: Guideline 81-1: Acute oral toxicity in rats

EPA IDENTIFICATION NUMBERS:

Caswell Number: 204
MRID Number: 418370-01

TEST MATERIAL: 4-Chlorophenoxyacetic acid

SYNONYMS: None

SPONSOR: Beatrice/Hunt-Wesson Inc., Fullerton, CA

STUDY NUMBER: HLA 00801531

TESTING FACILITY: Hazleton Laboratories America, Inc., Madison, WI

TITLE OF REPORT: Acute Exposure Oral Toxicity of 4-Chlorophenoxyacetic Acid in Rats

AUTHOR: S.M. Glaza

STUDY COMPLETED: 02/28/91

CONCLUSIONS: The estimated acute oral LD_{50} in male and female rats for 4-chlorophenoxyacetic acid was determined to be 2,703 mg/kg with 95% confidence limits of 2,191 to 3,335 mg/kg. Clinical signs of toxicity consisted of staggered gait, hypoactivity, absent pain reflex, red stained face, and dark stained urogenital area. All animals surviving to the end of the study exhibited body weight gain, except for one female rat at a dose of 700 mg/kg and one female rat at a dose of 2,000 mg/kg that demonstrated weight losses.

CORE CLASSIFICATION: Core supplementary. The method used for group assignment was not specified, and the reviewers question the validity of the estimated acute oral LD_{50} for females and sexes combined. It was not clear if cannibalized females were eliminated from the acute oral LD_{50} calculations. When cannibalized females are excluded from the mortality count at the highest dose level administered to females (2,000 mg/kg), 50% mortality was not achieved. Therefore, an acute oral LD_{50} for females cannot be determined.

TOXICITY CATEGORY: III--Caution

A. MATERIALS

1. Test Material

Test material: 4-Chlorophenoxyacetic acid
Purity: 99%
Physical description: White powder
Chemical no: 019401
CAS no: 122-88-3
pH: 6.0
Vehicle: 0.5N or 1.0N NaOH
Stability: Stable as a dry powder or aqueous solution from pH 1-13 at room temperature

2. Controls

Animals: None needed
Test Substance: None needed

3. Test Animals

Species: Rat
Strain: Crl:CDBR
Source: Charles River Laboratories, Inc., Portage, MI
Receipt date: 07/23, 08/13, 08/27, 09/24, and 10/08/90
Sex: Male and female
Numbers: 30 males and 25 females
Housing: Group housing by sex in groups of five. Animals identified by uniquely numbered ear tag. Acclimation period was at least 7 days.
Age: Young adult
Weight: At initiation: males (212-282 g), females (210-245 g)
Feeding: Feed and water provided ad libitum
Selection: Method for group assignment was not specified

4. Exposure

Route of administration: Oral gavage
Dose levels: Males: 700, 900, 1,100, 2,000, 2,600 and 3,200 mg/kg; females: 700, 900, 1,100, 1,500, and 2,000 mg/kg

B. TEST PERFORMANCE

Dose Range-finding Study

Initial doses of 700 or 900 mg/kg were administered by oral gavage to five male and five female rats at each dose level. Animals were fasted for 17-20 hours prior to dose administration. Rats were monitored for

clinical signs of toxicity at 1, 2.5, and 4 hours after dosing, and once daily throughout the 14-day observation period. Rats were monitored for mortality at 1, 2.5, and 4 hours after dosing, and twice daily throughout the 14-day observation period. A gross necropsy was performed on all animals.

LD_{50} Determination

Groups of five males and five females were fasted for 17-20 hours, and administered the test article by oral gavage. The dose levels for males consisted of 1,100, 2,000, 2,600, and 3,200 mg/kg. The dose levels for females consisted of 1,100, 1,500 and 2,000 mg/kg. Rats were monitored for clinical signs of toxicity at 1, 2.5, and 4 hours after dosing, and once daily throughout the 14-day observation period. Rats were monitored for mortality at 1, 2.5, and 4 hours after dosing, and twice daily throughout the 14-day observation period. Body weights were measured at test days 0, 7, and 14, and at death when survival exceeded one day. Gross necropsy was performed on all animals.

Statistics

Statistical analyses for LD_{50} values were performed using a computer program utilizing a modified Behrens-Reed-Muench Cumulant Method.

C. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

Dose Range-finding Study

No mortality was reported at doses of 700 and 900 mg/kg for either sex. At a dose of 700 mg/kg, no clinical signs of toxicity were noted for either sex. At a dose of 900 mg/kg, clinical signs of toxicity consisting of staggered gait, hypoactivity, absent pain reflex, red stained face, and dark stained urogenital area were noted for both sexes. Higher dose levels were added based on the absence of mortality at doses of 700 and 900 mg/kg.

LD_{50} Determination

No rats died at the 1,100 mg/kg level. A dose of 1,500 mg/kg was administered only to female rats; none died. Four females and one male died at the 2,000-mg/kg dose level; the mortality count includes 2 females and 1 male that were cannibalized because of the use of group housing. Doses of 2,600 and 3,200 mg/kg were administered only to male rats; 1/5 and 4/5 males died, respectively, none were cannibalized.

Clinical signs of toxicity consisted of staggered gait, hypoactivity, absent pain reflex, red stained face, and dark stained urogenital area. All animals surviving to the end of the study exhibited body weight gain, except for one female rat at a dose of 700 mg/kg and one female rat at a dose of 2,000 mg/kg, each demonstrated weight losses of 4 g on test days 7-14.

Necropsy of animals which died during the study revealed cannibalized areas, clear or dark red fluid in the stomach and/or urinary bladder, or no visible lesions. Terminal necropsy of surviving animals revealed enlarged submandibular lymph nodes, mottled red kidneys, or no visible lesions.

Based upon observed mortality, the estimated acute oral LD_{50} in males for 4-chlorophenoxyacetic acid was 2,811 mg/kg with 95% confidence limits of 2,334 to 3,385 mg/kg. The estimated acute oral LD_{50} for females was 1,796 mg/kg with 95% confidence limits of 1,519 to 2,122 mg/kg. The estimated acute oral LD_{50} for sexes combined was 2,703 mg/kg with 95% confidence limits of 2,191 to 3,335 mg/kg.

Tables were provided for incidence of mortality, clinical signs and body weights.

D. REVIEWERS' COMMENTS

Based on the acute oral LD_{50} in males and females of 2,703 mg/kg with 95% confidence limits of 2,191 to 3,335 mg/kg, the toxicity category was determined to be III--Caution.

This study was classified as Core Supplementary, using Guideline requirement 81-1. The method used for group assignment was not specified, and the reviewers question the validity of the estimated acute oral LD_{50} for females and sexes combined. It was not clear if cannibalized females were eliminated from the acute oral LD_{50} calculations. The female estimated acute oral LD_{50} of 1,796 mg/kg was based on mortality at the highest dosage level of 2,000 mg/kg. However, if the two cannibalized females are excluded from the mortality count of 4/5 females at the 2,000-mg/kg dose, only 2/5 female deaths could be attributed to treatment. Therefore, the dose levels chosen for the females may not have covered the 10% to 90% mortality range to allow for a more accurate determination of an LD_{50}. Higher doses are needed to more accurately determine the slope of the dose-response curve. Use of individual rather than group housing is advised in order to prevent cannibalism.

E. QUALITY ASSURANCE MEASURE

A signed Quality Assurance Statement, dated 2/28/91 was presented. A Good Laboratory Practice compliance statement was included.

F. CBI APPENDIX

None presented.

DATA EVALUATION REPORT

4-CHLOROPHENOXYACETIC ACID (4-CPA ACID)

Study Type: Mutagenicity: Gene Mutation in Cultured Mammalian Cells (Mouse Lymphoma Cells)

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll, B.S Date 2-27-92

Independent Reviewer Lynne T. Haber, Ph.D Date 2/27/92

QA/QC Manager Sharon Segal, Ph.D. Date 2/27/92

Contract Number: 68D10075
Work Assignment Number: 1-62
Clement Number: 91-195
Project Officer: James Scott

GUIDELINE SERIES 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

MUTAGENICITY STUDIES

EPA Reviewer: Jess Rowland — Signature: [signature]
Review Section II, Toxicology Branch (II)/HED — Date: 03/07/92
EPA Section Head: Clark Swentzel — Signature: [signature]
Review Section II, Toxicology Branch (II)/HED — Date: 3/10/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Gene mutation in cultured mammalian cells (mouse lymphoma cells)

EPA IDENTIFICATION Numbers:

Caswell Number: 204

MRID Number: 418370-04

TEST MATERIAL: 4-Chlorophenoxyacetic acid (4-CPA acid)

SYNONYMS: None provided; Cas number 122-88-3

SPONSOR: Beatrice/Hunt-Wesson, Inc., Fullerton, CA

STUDY NUMBER: 12447-0-431

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD

TITLE OF REPORT: Mutagenicity Test on 4-Chlorophenoxyacetic Acid in the L5178Y $TK^{+/-}$ Mouse Lymphoma Forward Mutation Assay with Independent Repeat

AUTHORS: R. R. Young and M. A. Cifone

REPORT ISSUED: March 5, 1991

CONCLUSIONS-EXECUTIVE SUMMARY: 4-Chlorophenoxyacetic acid (4-CPA acid) was evaluated for the potential to induce forward mutations at the $TK^{+/-}$ locus in L5178Y mouse lymphoma cells in two independently performed trials. Without S9 activation, 4-CPA was not mutagenic at doses of 100 to 4000 µg/mL; higher levels (5000 µg/mL) were severely cytotoxic. In the presence of S9 activation, nondose-related increases in the mutation frequency (MF) were obtained in both trials. Although there was a tendency for elevated MFs at doses ranging from 300 to 1600 µg/mL +S9, a doubling of the MF over concurrent controls was only seen at 600 µg/mL (Trial 1) and 1000 µg/mL (Trial 2). The evidence suggesting a mutagenic response, was, however, insufficient to conclude that 4-CPA acid was positive. We assess, therefore that the test material was not mutagenic in this in vitro mammalian cell assay.

STUDY CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84.2a) for genetic effects Category I, Gene Mutations.

A. MATERIALS:

1. Test Material: 4-Chlorophenoxyacetic acid (4-CPA acid)

Description: Off-white powder
Identification No.: CAS no. 122-88-3; batch or lot numbers were not provided
Purity: 99% (see Data Evaluation Record 91-194)
Receipt date: September 21, 1990
Stability: Not provided
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)--preliminary cytotoxicity test; Fischer's culture medium--mutation assay
Other provide information: The test material was stored at room temperature in the dark. Solutions of the test material were prepared on the day of use.

2. Control Materials:

Negative: Culture medium (RPMI 1640 medium supplemented with Pluronic F68, L-glutamine, sodium pyruvate, 10% horse serum, and antibiotics)--preliminary cytotoxicity assay only.

Solvent/final concentration:

Preliminary cytotoxicity test: DMSO/1%
Mutation assay: Fischer's 5% culture medium

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) at doses of 0.25 and 0.4 µL/mL and methyl methane sulfonate (MMS) at doses of 5.0 and 10.0 nL/mL were prepared in an unspecified solvent.

Activation (concentrations, solvent): 3-Methylcholanthrene (3-MCA) was prepared in an unspecified solvent to yield final concentrations of 2.5 and 4.0 µg/mL.

3. Activation: S9 derived from male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

The S9 liver homogenate (Lot number 0282) was prepared by Molecular Toxicology, Inc., Annapolis, MD. Prior to use, the S9 fraction was characterized for its ability to convert 3-MC to a mutagenic form using mouse lymphoma cells.

S9 mix composition:

Component	Final concentration/mL in cultures
NADP	3 mM
Isocitrate	15 mM
S9 homogenate	15 µL/mL

4. Test Cells: Mammalian cells in culture

__x__ mouse lymphoma L5178Y cells
_____ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Yes.
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

__x__ thymidine kinase (TK)
selection agent: (give concentration)
_____ bromodeoxyuridine (BrdU)
_____ fluorodeoxyuridine (FdU)
3 µg/mL trifluorothymidine (TFT)

_____ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
selection agent: (give concentration)
_____ 8-azaguanine (8-AG)
_____ 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
selection agent: (give concentration)
_____ ouabain

_____ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

(a) Cytotoxicity assay: Ten doses (2, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 µg/mL) were evaluated in the presence and absence of S9 activation.

(b) Mutation assay:

(1) Nonactivated conditions:

Initial assay: 50, 100, 300, 600, 1000, 1300, 1600, 2000, 3000, 4000, and 5000 µg/mL.

Confirmatory assay: 100, 500, 1000, 1300, 1600, 2000, 2500, 3000, 3500, and 4000 µg/mL.

(2) S9-activated conditions:

Initial assay: As above for the initial nonactivated assay.

Confirmatory assay: 10, 50, 100, 250, 500, 750, 1000, 1300, 1600, and 2000 µg/mL.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound for:
4 hours (nonactivated) 4 hours (activated)

(b) Cells exposed to positive controls for:
4 hours (nonactivated) 4 hours (activated)

(c) Cells exposed to negative and/or solvent controls for:
4 hours (nonactivated) 4 hours (activated)

(d) After washing, cells cultured for 2 days (expression period) before cell selection

(e) After expression, cells cultured for 10 to 14 days in selection medium to determine numbers of mutants and for 10 to 14 days without selection medium to determine cloning efficiency.

2. Statistical Methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria:

(a) Assay validity: For the assay to be considered valid, the following criteria must be satisfied: (1) the absolute cloning efficiency (CE) of the negative control should be 60-130%; (2) the mutation frequency (MF) of the solvent control must be between 20 and 90 mutant colonies x 10^{-6}; and (3) the MF of the positive controls must be ≥200 mutant colonies x 10^{-6}.

(b) Positive response: The test material was considered positive if it induced a reproducible dose-related or toxicity-related increase in the MF that exceeded 2 times the MF of the concurrent background control.

4. Protocol: None provided.

C. REPORTED RESULTS:

1. Test Material Solubility: The test material was reported to be insoluble in deionized H_2O at ≥100 mg/mL and soluble at 343 mg/mL in DMSO. Upon addition to culture medium, ≥1.7 mg/mL of the test

material in DMSO formed a white precipitate and reduced the pH of the medium to 5.7. Based on these findings, DMSO was selected as the solvent to prepare a primary stock solution of 60 mg/mL. The primary stock was diluted in culture medium to yield 5.5 mg/mL and neutralized with NaOH to pH 6.8. Complete dissolution of the test material resulted from the pH adjustment. The 5.5 mg/mL solution was further diluted with culture medium to obtain the final stock concentration (2.0 mg/mL) used in the cytotoxicity assay.

2. Cytotoxicity Assays: Results from the cytotoxicity assay conducted with 2 to 1000 µg/mL 4-CPA acid indicated that the high dose was only slightly cytotoxic without S9 activation (74.3% relative survival) and moderately cytotoxic with S9 activation (36.0% relative survival). Accordingly, the mutation assay was initiated with higher starting concentrations (50 to 5000 µg/mL +/-S9). Since the preliminary findings revealed that the test material was soluble at a neutral pH, the solvent was changed to Fischer's 5% medium; appropriate pH adjustments were made.

3. Mutation Assay:

   (a) Nonactivated conditions: Representative results from the initial and confirmatory mutation assays with 4-CPA acid are presented in Table 1. In the initial trial, relative suspension growth (RSG) was 29.1% for the highest cloned treatment group (3000 µg/mL); higher levels (4000 and 5000 µg/mL) reduced RSG to ≤3.7%. RSG for the remaining concentrations was ≥72.4%. There was a slight increase in total mutant colonies and the MF at 3000 µg/mL. However, the increase was <2-fold over background and was confined to this dose. Results from the confirmatory trial were in general agreement with the initial findings showing that nonactivated 4-CPA acid was not mutagenic over a concentration range of 100 to 4000 µg/mL. The ~2-fold over background increase in the MF (106.3×10^{-6}) at 4000 µg/mL fell within the generally accepted spontaneous MF range for mouse lymphoma cells (i.e., 15-110 mutants/10^6 survivors).[1] Hence, our reviewers did not consider this finding to be suggestive of a mutagenic response. The two nonactivated positive controls (0.25 and 0.40 µL/mL EMS and 5.0 and 10.0 nL/mL MMS) induced marked and dose-related increases in mutation at the $TK^{+/-}$ locus.

   (b) S9-Activated conditions: In the presence of S9 activation, <10% of the cells survived exposure to the five highest doses (1600 to 5000 µg/mL) evaluated in the initial trial.

   Survival at the remaining levels (50 to 1300 µg/mL) was concentration-dependent and ranged from 7.2% at 1300 µg/mL to 123.4% at 50 µg/mL. Our reviewers noted, however, that the cytotoxicity

[1]Caspary, W.J., Lee, Y.J., Poulton, S., Myhr, B.C., Mitchell, A.D., Rudd, C.J. (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Quality-control guidelines and response categories. Environ. Mol. Mutagen 12:19-36.

TABLE 1. Representative Results of the Nonactivated Mouse Lymphoma Forward Mutation Assays with 4-Chlorophenoxyacetic Acid

Substance	Dose/mL	Percent Relative Suspension Growth	Mutant Colonies[a]	Viable Colonies[a]	Percent Relative Cloning Efficiency[a]	Percent Relative Total Growth[a]	Mutation Frequency[a,b] 10^{-6}
Solvent Control							
Fischer's 5%	--	100.0[c]	109	526	100.0	100.0	41.8 (41.4)
	--	100.0[d]	145	546	100.0	100.0	53.8 (52.2)
Positive Control[e]							
Ethyl methsnesulfonate	0.25 µL	79.6[c,f]	1073	409	77.8[f]	61.9	524.7[g]
	0.25 µL	82.3[d,f]	1201	510	93.5[f]	76.9	471.0[g]
Methyl methanesulfonate	5.00 nL	107[c,f]	544	444	84.4[f]	90.3	245.0[g]
	5.00 nL	101[d,f]	627	510	93.5[f]	94.5	245.9[g]
Test Material							
4-Chlorophenoxyacetic acid	2000 µg[h]	72.4[c]	106	521	99.0	71.7	40.7
	3000 µg[i]	29.1	169	465	88.4	25.7	72.7
	3000 µg[h]	52.4[d]	203	474	86.9	45.5	85.7
	3500 µg	34.0	195	437	80.1	27.2	89.2
	4000 µg	20.6	238	448	82.1	16.9	106.3

[a]Average values for triplicate solvent control cultures were calculated by our reviewers. Single cultures were used for the positive controls and the test material doses.

[b]Mutation Frequency (MF) = $\frac{\text{Mutant Colonies}}{\text{Viable Colonies}} \times 2\text{x}10^{-4}$; MFs in () were calculated by our reviewers using this formula.

[c]Results from the initial trial.
[d]Results from the confirmatory trial.
[e]Two levels of each positive control were assayed; results from the lower doses were selected as representative.
[f]Calculated by our reviewers.
[g]Exceeded the reporting laboratory's criterion for a positive response (i.e., MF$\geq$83.6x10^{-6}--initial trial; $\geq$107.5x10^{-6}--confirmatory trial).
[h]Results for lower doses (50, 100, 1000, 1300, and 1600 µg/mL--initial trial and 100, 1000, 2000 and 2500 µg/mL--confirmatory trial) did not suggest a mutagenic effect.
[i]Higher levels (4000 and 5000 µg/mL) were too cytotoxic to clone.

curve was relatively steep at intermediate doses. RSG was 30.7% at 600 µg/mL as compared to 82.5% at 300 µg/mL; relative total growth (RTG) was similarly affected (Table 2). Mutant colonies and MFs were elevated at all levels; however, at 300 and 600 µg/mL, ≥1.8-fold increases over background were calculated by our reviewers; the value for 600 µg/mL (123.0×10^{-6}) exceeded the reporting laboratory's minimum requirement for a positive result (113.4×10^{-6}). In the repeat assay, similar evidence of a steep cytotoxicity curve was observed between 250 and 500 µg/mL; however, the 250-µg/mL treatment group was not plated, thereby, limiting the comparative evaluation of the data from both assays. Nevertheless, a 2-fold increase in mutation was obtained at 1000 µg/mL; the MFs were also increased at 500 and 750 µg/mL (≥1.6-fold higher than control). In an attempt to further elucidate the overall S9-activated results, the data from relevant experimental points in both assays were combined by our reviewers and are shown in Table 3. Presentation of the combined results clearly demonstrates that the cytotoxicity data from both assays were in good agreement and that reductions in RSG and RTG were dose-related. Table 3 also illustrates the sharp decline in cytotoxicity between the 250/300-µg/mL and the 500/600-µg/mL treatment groups. However, a dose-related mutagenic response was not uncovered. The study authors stated that the response was consistent with normal assay variation and, therefore, concluded that 4-CPA acid was not mutagenic in mouse lymphoma cells.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that in the absence or presence of S9 activation, 4-CPA acid was not mutagenic in mouse lymphoma cells. Although MFs were elevated at doses ranging from 300 to 1600 µg/mL +S9, a doubling of the MF was only seen at 600 µg/mL (Trial 1) and 1000 µg/mL (Trial 2). These findings are, however, not sufficient to conclude that 4-CPA induced a mutagenic response in mouse lymphoma cells.

By contrast, the nonactivated positive controls (0.25 and 0.4 µL/mL EMS and 5 and 10 nL/mL MMS) and S9-activated positive control (2.5 and 4.0 µg/mL) induced dose-related mutagenic effects indicating that all trials were adequately sensitive to detect a genotoxic response.

We conclude, therefore, that appropriate concentrations of 4-CPA were tested and that the results provided no compelling evidence of a positive response in this in vitro mammalian cell test system.

TABLE 2. Representative Results of the S9-Activated Mouse Lymphoma Forward Mutation Assays with 4-Chlorophenoxyacetic Acid

Substance	Dose/mL	Percent Relative Suspension Growth	Mutant Colonies[a]	Viable Colonies[a]	Percent Relative Cloning Efficiency[a]	Percent Relative Total Growth[a]	Mutation Fraquency[a,b] 10^{-6}	Fold Increase[c]
Solvent Control								
Fischer's 5X	--	100[d]	148	523	100.0	100.0	56.7 (56.6)	--
	--	100[e]	202	565	100.0	100.0	72.0 (71.5)	--
Positive Control[f]								
3-Methylcholanthrene	2.5 µg/mL	68.1[d,g]	837	401	76.7	52.3[g]	417.5[h]	7.4
	2.5 µg/mL	69.6[e,g]	1202	446	78.9	54.9[g]	539.0[h]	7.5
Test Material								
4-Chlorophenoxyacetic acid	100 µg/mL[i]	99.4[d]	172	550	105.2	104.6	62.5	1.1
	300 µg/mL	82.5	257	516	98.7	81.4	99.6	1.8
	600 µg/mL	30.7	233	379	72.5	22.3	123.0[h]	2.2
	1000 µg/mL	12.5	199	459	87.8	11.0	86.7	1.5
	1300 µg/mL[j]	7.2	204	447	85.5	6.2	91.3	1.6
	100 µg/mL[i]	101.2[e]	214	463	81.9	82.9	92.4	1.3
	250 µg/mL	81.6	NC[k]	--	--	--	--	--
	500 µg/mL	36.3	317	538	95.2	34.6	117.8	1.7
	750 µg/mL	22.2	247	427	75.5	16.8	115.7	1.6
	1000 µg/mL	14.8	308	437	77.3	11.4	141.0	2.0
	1300 µg/mL	10.5	266	517	91.5	9.6	102.9	1.4
	1600 µg/mL[j]	6.2	305	514	90.9	5.6	118.7	1.7

[a]Average values for triplicate solvent control cultures were calculated by our reviewers. Single cultures were used for the positive controls and the test material doses.

[b]Mutation Frequency (MF) = $\frac{\text{Mutant Colonies}}{\text{Viable Colonies}} \times 2\text{x}10^{-4}$; MFs in () were calculated by our reviewers using this formula.

[c]Fold Increase = $\frac{\text{MF Test Dose}}{\text{MF Solvent Control}}$; calculated by our reviewers.

[d]Results from the initial trial.
[e]Results from the confirmatory trial.
[f]Two levels of the positive control were assayed; results from the lower dose were selected as representative.
[g]Calculated by our reviewers.
[h]Exceeded the reporting laboratory's criterion for a positive response (i.e., MF≥113.4x10^{-6}--initial trial; 144.0x10^{-6}--confirmatory trial).
[i]Results for lower levels (50 µg/mL--initial trial and 10 µg/mL--confirmatory trial) were negative.
[j]Higher doses (1600, 3000, 4000 and 5000 µg/mL--initial trial and 2000 µg/mL--confirmatory assay) were too cytotoxic to clone.
[k]NC = Not cloned.

TABLE 3. Representative Combined Results of the Two S9-Activated Mouse Lymphoma Forward Mutation Assays with 4-Chlorophenoxyacetic Acid

Substance	Dose/mL	Percent Relative Suspension Growth	Mutant Colonies	Viable Colonies	Percent Relative Cloning Efficiency	Percent Relative Total Growth	Mutation Frequency[a] 10^{-6}	Fold Increase[b]
Solvent Control								
Fischer's 5%	--	100[c]	148	523	100.0	100.0	56.6	--
	--	100[d]	202	565	100.0	100.0	71.5	--
Test Material								
4-Chlorophenoxyacetic acid	250 µg/mL	81.6[d]	NC[e]	--	--	--	--	--
	300 µg/mL	82.5[c]	257	516	98.7	81.4	99.6	1.8
	500 µg/mL	36.3[d]	317	538	95.2	34.6	117.8	1.7
	600 µg/mL	30.7[c]	233	379	72.5	22.3	123.0	2.2
	750 µg/mL	22.2[d]	247	427	75.5	16.8	115.7	1.6
	1000 µg/mL[f]	12.5[c]	199	459	87.8	11.0	86.7	1.5
	1000 µg/mL[f]	14.8[d]	308	437	77.3	11.4	141.0	2.0

[a]Mutation Frequency (MF) = $\frac{\text{Mutant Colonies}}{\text{Viable Colonies}} \times 2\text{x}10^{-4}$.

[b]Fold Increase = $\frac{\text{MF Test Dose}}{\text{MF Solvent Control}}$; calculated by our reviewers.

[c]Results from the initial trial.
[d]Results from the confirmatory trial.
[e]NC = Not cloned.
[f]Percent Relative Total Growth for higher levels (1300 µg/mL--initial trial and 1300 and 1600 µg/mL--confirmatory trial) was <10%.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement from the reporting laboratory was signed and dated March 5, 1991).

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 11-21.

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84.2a) for genetic effects Category I, Gene Mutations.

APPENDIX A

MATERIALS AND METHODS
CBI pp. 11-21

Pages 21-30 - *Access to FIFRA health and safety data is restricted under FIFRA section 10(g)*

CASWELL FILE

DOC 920166

FINAL

009417

DATA EVALUATION REPORT

4-Chlorophenoxyacetic Acid

Study Type: Mutagenicity: Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Lynne T. Haber, Ph.D. Date 2/24/92

Independent Reviewer Nancy E. McCarroll, B.S. Date 2/24/92

QA/QC Manager Sharon Segal, Ph.D. Date 2/24/92

Contract Number: 68D10075
Work Assignment Number: 1-62
Clement Number: 91-193
Project Officer: James Scott

GUIDELINE SERIES 84: MUTAGENICITY
SALMONELLA

EPA Reviewer: Jess Rowland — Signature: Jess Rowland
Review Section II, Toxicology Branch (II)/HED — Date: 03/02/92
EPA Section Head: Clark Swentzel — Signature: R. Clark Swentzel
Review Section II, Toxicology Branch (II)/HED — Date: 3/10/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay

EPA IDENTIFICATION Numbers:

Caswell Number: 204

MRID Number: 418370-02

TEST MATERIAL: 4-Cholorophenoxyacetic acid

SYNONYMS: None provided; CAS no. 122-88-3

SPONSOR: Beatrice/Hunt-Wesson, Inc., Fullerton, CA

STUDY NUMBER: 12447-0-401R

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD

TITLE OF REPORT: Mutagenicity Test on 4-Chlorophenoxyacetic Acid in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test) with a Confirmatory Assay

AUTHOR: Lawlor, T. E.

REPORT ISSUED: December 24, 1990

CONCLUSIONS--EXECUTIVE SUMMARY: Under the conditions of two independently performed Salmonella typhimurium/mammalian microsome plate incorporation assays, 4-chlorophenoxyacetic acid (4-CPA) was assayed at S9-activated and nonactivated doses ranging from 100 to 5000 µg/plate. Nonactivated levels ≥3330 µg/mL were cytotoxic; no cytotoxicity was seen in the presence of S9 activation. The test compound did not induce a mutagenic response in S. typhimurium strains TA1535, TA1537, TA1538, TA98, or TA100 either in the absence or the presence of microsomes derived from Aroclor 1254-induced rat livers S9. Based on these findings, it was concluded that 4-CPA was tested over an appropriate range of concentrations with no evidence of a mutagenic effect. The study, therefore, satisfies Guideline requirements for genetic effects Category I, Gene Mutations.

STUDY CLASSIFICATION: The study is acceptable.

A. MATERIALS:

1. Test Material: 4-Chlorophenoxyacetic acid (4-CPA acid)

Description: Off-white powder
Lot number: Not reported
Purity: 99%
Receipt date: September 21, 1990
Stability: Not reported
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored at room temperature. The frequency of dosing solution preparation was not reported.

2. Control Materials:

Solvent/final concentration: DMSO/50µl per plate

Positive:

Nonactivation:

Sodium azide	2	µg/plate TA100, TA1535
2-Nitrofluorene	1	µg/plate TA98, TA1538
ICR 191	2	µg/plate TA1537

Activation:

2-Aminoanthracene	2.5	µg/plate all strains

3. Activation: S9 derived from male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The rat liver S9 homogenate was purchased from Molecular Toxicology, Inc., Annapolis, MD. The batch used in the assay (0311) was reported to contain 39.4 mg protein/mL.

S9 mix composition:

Component:	Volume/mL
Water	0.70 mL
1 M Sodium phosphate buffer (pH 7.4)	0.10 mL
0.25 M Glucose 6-phosphate	0.02 mL
0.10 M NADP	0.04 mL
0.825 M KCl/0.2 M $MgCl_2$	0.04 mL
S9	0.10 mL (10% final concentration)

4. Test Organism Used: S. typhimurium strains
____ TA97 _x_ TA98 _x_ TA100 ____ TA102 ____ TA104
_x_ TA1535 _x_ TA1537 _x_ TA1538
list any others:

Test organisms were properly maintained: Yes.
Checked for appropriate genetic markers (rfa mutation, R factor): Yes.

5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Ten doses (6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg/plate) were evaluated with and without S9 activation in S. typhimurium strain TA100. A single plate was used, per dose, per condition.

(b) Mutation assays:

(1) Initial: Six doses (100, 333, 667, 1000, 3330, and 5000 µg/plate) were evaluated in triplicate in the presence and absence of S9 activation; all tester strains were used.

(2) Confirmatory: As above.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
_x_ Standard plate test
____ Pre-incubation (____) minutes
____ "Prival" modification
____ Spot test
____ Other (describe)

2. Protocol:

(a) Preliminary cytotoxicity/mutation assays: Similar procedures were used for the preliminary cytotoxicity and the mutation assays.

At least 0.5×10^8 cells (0.1 mL of a $\geq 0.5 \times 10^9$ cells/mL late log phase culture) of the appropriate tester strain and 50 µL of the appropriate test material dose, solvent, or positive controls were added to tubes containing 2.5-mL volumes of molten top agar. Sufficient water was added to the top agar in the nonactivated tests to ensure that equivalent concentrations of amino acid supplements were available under the nonactivated and S9-activated conditions. For the S9-activated assay, 0.5 mL of the S9-cofactor mix was added to 2 mL of the top agar. Tester strains, test solutions, and control solutions were added as described. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E plates, and incubated at 37±2°C for 48±8 hours. At the end of incubation, plates were either scored immediately for revertant colonies or were refrigerated and subsequently counted. Means and standard devia-

tions for the mutation test were determined from the counts of triplicate plates per strain, per dose, per condition.

(b) Sterility controls: The sterility of the highest test dose and the S9 mix were determined.

3. Evaluation Criteria:

(a) Assay validity: In order for the assay to be considered valid, it must meet the following criteria: (1) the presence of the appropriate genetic markers must be verified; (2) tester strain culture titers must be ≥0.5 x 10^9 cells/mL; (3) positive control values must show at least a tripling in the mean number of revertants for each strain (+/- S9); and (4) at least three nontoxic doses of the test compound should be assayed. In addition, the spontaneous revertants for each strain should fall into the following range: TA98, 8-60; TA100, 60-240; TA1535, 4-45; TA1537, 2-25; and TA1538, 3-35.

(b) Positive response: The test material was considered positive if it caused a dose-related increase in the mean number of revertants per plate of at least one strain. This increase must be at least two-fold in strains TA98 and TA100, and at least three-fold in strains TA1535, TA1537, and TA1538.

C. REPORTED RESULTS

1. Preliminary Cytotoxicity Assay: Ten doses of the test material ranging from 6.67 to 5000 µg/plate were evaluated with and without S9 activation using strain TA100. No revertants survived exposure to the highest nonactivated dose (5000 µg/plate). At 3330 µg/plate -S9, the number of revertants/plate was reduced by 32% and a slight reduction in the background lawn of growth was observed. No cytotoxicity was observed at any S9-activated level. Based on these findings, the concentration range selected for the mutation assay was 100-5000 µg/plate +/- S9.

3. Mutation Assay: In agreement with the results of the preliminary cytotoxicity assay, 5000 µg/plate -S9 caused severe cytotoxicity in both the initial and confirmatory trials. Similarly, reduced revertant colonies and background lawns of growth were seen in all strains at 3330 µg/plate -S9. The S9-activated test material was not cytotoxic. Results from the initial and confirmatory trials further indicated that 4-CPA was not mutagenic in any tester strain in the presence or absence of S9 activation (Tables 1 and 2). In contrast, all strains responded to the appropriate nonactivated and S9-activated positive controls in both trials. From the overall findings, the study author concluded that 4-CPA was not mutagenic in this test system.

TABLE 1: Representative Results of the Initial Salmonella typhimurium/Mammalian Microsome Mutation Assay with 4-Chlorophenoxyacetic Acid

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethyl sulfoxide	50 µL	-	12±5	10±2	18±4	15±3	80±5
	50 µL	+	12±2	8±2	22±7	28±3	99±2
Positive Controls							
Sodium azide	2 µg	-	295±111	--	--	--	511±21
ICR-191	2 µg	-	--	251±13	--	--	--
2-Nitrofluorene	1 µg	-	--	--	172±27	151±30	--
2-Aminoanthracene	2.5 µg	+	81±7	97±15	989±69	538±6	621±70
Test Material							
4-Chlorophenoxyacetic Acid	1000 µg[b]	-	12±2	6±1	10±6	18±2	85±9
	3330 µg[c]	-	6±4	3±1	8±3	4±5	33±18
	5000 µg[b]	+	11±2	8±5	17±4	31±2	91±19

[a]Means and standard deviations of the counts from triplicate plates.

[b]Results for lower doses (100, 333, and 667 µg/plate -S9, and 100, 333, 667, 1000, and 3330 µg/plate +S9) did not suggest a mutagenic effect.

[c]The highest nonactivated dose (5000 µg/plate) was severely cytotoxic.

TABLE 2: Representative Results of the Confirmatory Salmonella typhimurium/Mammalian Microsome Mutation Assay with 4-Chlorophenoxyacetic Acid

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethyl sulfoxide	50 µL	-	14±3	8±2	10±3	24±9	99±7
	50 µL	+	12±3	8±2	17±3	34±6	99±13
Positive Controls							
Sodium azide	2 µg	-	533±36	--	--	--	622±63
ICR-191	2 µg	-	--	354±82	--	--	--
2-Nitrofluorene	1 µg	-	--	--	230±72	171±34	--
2-Aminoanthracene	2.5 µg	+	147±7	128±12	1081±30	961±41	986±76
Test Material							
4-Chlorophenoxyacetic Acid	1000 µg[b]	-	16±5	9±2	15±1	18±6	77±6
	3330 µg[c]	-	5±4	2±1	4±5	0	11±7
	5000 µg[b]	+	10±7	3±1	15±4	23±2	83±11

[a]Means and standard deviations of the counts from triplicate plates.

[b]Results for lower doses (100, 333, and 667 µg/plate -S9, and 100, 333, 667, 1000, and 3330 µg/plate +S9) did not suggest a mutagenic effect.

[c]The highest nonactivated dose (5000 µg/plate) was severely cytotoxic.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study author's interpretation of the data was correct. The test material was assayed to cytotoxic levels without S9 activation (≥3330 µg/plate) and to an acceptably high noncytotoxic dose with S9 activation, but failed to induce a mutagenic effect in a well-controlled study. In addition, the response of all tester strains to the appropriate direct-acting or promutagenic positive controls indicated that the assay had an adequate level of sensitivity to detect mutagenesis. It was concluded, therefore, that 4-CPA was negative in this microbial test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated December 31, 1990.)

F. CBI APPENDICES: Appendix A, Materials and Methods, CBI pp. 14-23.

APPENDIX A

MATERIALS AND METHODS
CBI pp. 14-23

Pages 40-49 - *Access to FIFRA health and safety data is restricted under FIFRA section 10(g)*

DATA EVALUATION REPORT

4-CHLOROPHENOXYACETIC ACID (4-CPA ACID)

Study Type: Mutagenicity: In Vivo Micronucleus Assay in Mice

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll, B.S Date 2-27-92

Independent Reviewer Lynne Haber, Ph.D. Date 2/27/92

QA/QC Manager Sharon Segal, Ph.D. Date 2/27/92

Contract Number: 68D10075
Work Assignment Number: 1-62
Clement Number: 91-194
Project Officer: James Scott

MUTAGENICITY STUDIES

EPA Reviewer: Jess Rowland
Review Section II,
Toxicology Branch II/HED (H-7509C)
Signature: Jess Rowland
Date: 2/10/92

EPA Section Head: Clark Swentzel
Review Section II,
Toxicology Branch II/HED (H-7509C)
Signature: K. Clark Swentzel
Date: 3/10/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo micronucleus assay in mice

EPA IDENTIFICATION Numbers:

Caswell Number: 204

MRID Number: 418370-03

TEST MATERIAL: 4-Chlorophenoxyacetic acid (4-CPA acid)

SYNONYMS: None provided; CAS no. 122-88-3

SPONSOR: Beatrice/Hunt-Wesson, Inc., Fullerton, CA

STUDY NUMBER: 12447-0-455PO

TESTING FACILITY: Hazleton Washington, Inc., Kensington, MD

TITLE OF REPORT: Mutagenicity Test On 4-Chlorophenoxyacetic Acid In Vivo Micronucleus Assay

AUTHOR: H. Murli

REPORT ISSUED: February 7, 1991

CONCLUSIONS--EXECUTIVE SUMMARY: The single oral gavage administration of 450, 900, or 1800 mg/kg 4-chlorophenoxyacetic acid (4-CPA acid) to male or female mice did not cause a significant increase in the frequency of micronucleated polychromatic erythrocytes (MPEs) in bone marrow cells harvested 24, 48, or 72 hours posttreatment. Deaths and cytotoxic effects on the target organ (bone marrow cells) were seen in high-dose males and females. Based on these findings, we assess that 4-CPA acid was adequately tested and found to be nonclastogenic in the mouse micronucleus assay. The study, therefore, satisfies Guideline requirements for genetic effects Category II, Structural Chromosomal Aberrations.

STUDY CLASSIFICATION: Acceptable. The study satisfies data Guideline requirements (§84-26) for a mouse micronucleus assay.

A. MATERIALS:

1. Test Material: 4-Chlorophenoxyacetic acid (4-CPA acid)

Description: Off-white powder
Identification Number: CAS no. 122-88-3; batch or lot numbers were not provided
Purity: 99%
Receipt date: September 21, 1990
Stability: Not provided
Contaminants: None listed
Solvents used: 0.5N NaOH, 1.0 N NaOH, 5% HCl, sterile deionized water (DH_2O)
Other provided information: Owing to the solubility properties of the test material, the above series of solvents were used as follows: 198 mg 4-CPA acid were dissolved in 1.85 mL of 0.5N NaOH (1.0N NaOH was also used because of the limited solubility of 4-CPA acid in 0.5N NaOH); 0.04 mL of 5% HCl were added to adjust the pH to 6.0; and the solution was brought to volume with DH_2O to yield a final concentration of 94.3 mL/mL.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: 0.5N NaOH, 1.0N NaOH, 5% HCl, DH_2O at a dosing volume of 20 mL/kg was administered by oral gavage.

Positive/final concentration/route of administration: Cyclophosphamide (CP) was dissolved in DH_2O and administered by oral gavage at 80 mg/kg; dosing volume = 10 mL/kg.

3. Test Compound:

Route of administration: Oral gavage

Dose levels used: 450, 900, 1800 mg/kg (5 males and 5 females per dose, per sacrifice time)

Note: Dose selection was based on the findings of an acute study indicating that the oral gavage LD_{50} was 2000-2600 mg/kg (male mice) and 1100-2000 mg/kg (female mice).

Secondary group: An additional group of animals (10/sex) received the high dose of the test material. Animals in the secondary group were used only to replace animals that died in the primary group.

4. Test Animals:

(a) Species mouse Strain ICR Age (at dosing) 8 weeks and 1 day
Weight range: 23.7-33.9 g (males); 20.0-26.3 g (females)
Source: Harlan Sprague-Dawley, Inc., Frederick, MD

(b) No. animals used per dose: 15 males; 15 females

Note: Dosing was based on individual body weights; these data were not provided.

(c) Properly maintained? yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x 24 hr x 48 hr x 72 hr

(b) Vehicle control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): x 24 hr _____ 48 hr
_____ 72 hr

(c) Positive control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): x 24 hr _____ 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

x bone marrow _____ others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: 1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: 1000

3. Details of Slide Preparation: At 24, 48, and 72 hours after administration of the test material, the appropriate groups of animals were sacrificed by CO_2 asphyxiation. Sacrifice time for the vehicle and positive control groups was 24 hours. Bone marrow cells were aspirated from both tibiae, mixed with fetal calf serum, and spread onto slides. Prepared slides were fixed in methanol, stained with May-Grunwald and Giemsa solutions, coverslipped, coded, and scored.

4. Statistical Methods: The results were evaluated for statistical significance at $p<0.05$ using an analysis of variance (ANOVA) and Tukey's Studentized range test on transformed data (square root arsine proportion).

5. Evaluation Criteria: The test material was considered positive for micronuclei induction if a significant ($p<0.05$) increase in micronucleated polychromatic erythrocytes (MPEs) compared to the solvent control was seen, and the response was dose-related.

C. REPORTED RESULTS:

1. Animal Observations: Animals were observed immediately following dosing and periodically thereafter. Languidness was apparent in "some" high-dose animals immediately following dosing. Deaths occurring in the 1800-mg/kg treatment group were as follows: 5 males and 11 females (~16 hours posttreatment), 2 males and 3 females (21 hours posttreatment) and 2 males (prior to the 24-hour harvest). Other signs of compound toxicity reported at 16- or 21-hours posttreatment in the high-dose group included languidness and prostration. With the exception of one male, high-dose group survivors appeared normal by 48 hours. No toxic signs or death were seen in the low- (450 mg/kg) or the mid- (900 mg/kg) treatment groups. Owing to the high mortality rates, surviving females in the 1800-mg/kg group were reassigned to different sacrifice time to ensure adequate sample sizes at each harvest interval.

2. Micronucleus Assay: Representative findings from the micronucleus assay are shown in Table 1. No significant increases in the frequency of micronucleus induction was seen in bone marrow cells of male and female mice sampled 24, 48, or 72 hours postexposure to the three selected doses of 4-CPA acid. However, PCE:NCE ratios were depressed in the high-dose males 48 and 72 hours following treatment and in high-dose females at all sampling intervals. This finding indicated that 1800 mg/kg had an adverse effect on hematopoiesis.

   Based on the overall results, the study author concluded that 4-CPA acid was negative in this in vivo study.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: Our assessment is in agreement with the study author that 4-CPA acid was not clastogenic in this in vivo assay. The evidence of overt compound toxicity in conjunction with an adverse effect on bone marrow stem cells indicated that the high level (1800 mg/kg) selected for the study adequately demonstrated that the maximum tolerated dose was achieved.

Additionally, the sensitivity of the test system to detect a genotoxic response in male and female mouse bone marrow cells was shown by the significant ($p<0.05$) results obtained with the positive control (80 mg/kg CP).

TABLE 1. Representative Results of the Micronucleus Assay in Mice with Treated with 4-Chlorophenoxyacetic Acid (4-CPA Acid)

Substance	Dose/kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group	Mean Percent MPEs ±S.E.	Mean PCE/NCE Ratio ±S.E.
Vehicle Control								
0.5N NaOH, 1.0N NaOH, 5% HCl, deionized water	20 mL	24	M	5	5000	0	0.00±0.00	0.92±0.13
		24	F	5	5000	5	0.10±0.06	1.31±0.12
Positive Control								
Cyclophosphamide	80 mg	24	M	5	5000	57	1.14±0.13*	0.63±0.08
		24	F	5	5000	93	1.86±0.43*	1.18±0.12
Test Material								
4-CPA acid	1800 mg[b]	24	M	5	5000	0	0.00±0.00	0.93±0.23
		24	F	5	5000	3	0.06±0.04	0.57±0.22
		48	M	5	5000	3	0.06±0.04	0.54±0.08
		48	F	3	3000	1	0.03±0.03	0.42±0.11
		72	M	5	5000	3	0.06±0.02	0.51±0.17
		72	F	3	3000	4	0.13±0.03	0.44±0.16

[a]Time after compound administration.
[b]Deaths occurring in the high-dose group included 9 males and 14 females; signs of languidness and prostration were also seen in the 1800-mg/kg treatment group. Results for the low- (450 mg/kg) and mid- (900 mg/kg) dose groups did not suggest a clastogenic effect.

*Significantly higher ($p<0.05$) than the correeponding vehicle control by ANOVA.

Abbreviations used:

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated February 7, 1991).

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 12-16.

CORE CLASSIFICATION: Acceptable. The study satisfies the data Guideline requirements (§84-26) for a mouse micronucleus assay.

APPENDIX A

MATERIALS AND METHODS
CBI pp. 12-16

Pages 58-62 - *Access to FIFRA health and safety data is restricted under FIFRA section 10(g)*

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Chemical:	**4-CPA**
PC Code:	**019401**
HED File Code	**13000 Tox Reviews**
Memo Date:	**04/06/92**
File ID:	**TX009417**
Accession Number:	**412-02-0004**

HED Records Reference Center
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